

IN VITRO CALLUS PRODUCTION FROM LEAVES OF GYMNEMA SYLVESTRE R. BR

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ABSTRACT: *Successful callusing was obtained from leaf segments of *Gymnema sylvestre* R. Br. Cultures aseptically on MS medium with various concentrations of growth regulators.*

INTRODUCTION

Gymnema sylvestre (Asclepiadaceae), commonly known as Small Indian Ipecacuanha, Periploca of woods or Gurmar is a stout woody climber which grows in Central and Southern India. The plant has been described in the Hindu material medica as anti-periodic, stomachic and diuretic. Susruta described the plant as a “destroyer of **madhumeha**” (glycosuria) and other urinary disorders. About a hundred years ago, Edgeworth noticed that when the leaves of this plant were chewed, perception of the sweetness of cane sugar and all saccharine substances was abolished. This was later confirmed by Hooper who discovered that the leaf also had the remarkable property of completely masking the bitter taste of substances such as quinine. The peculiar gustatory effects produced by the plant last for one or two hours (Chopra et al. 1958).

Diabetes mellitus is a common metabolic disorder of human beings. It is global in distribution, affecting 2 – 6 per cent population of the world. Banting and best solved the problem of diabetes to a great extent by extracting the insulin from pancreas. But long-term complications are

not preventable by the present mode of therapy. Though insulin is available in various forms, prick pain, risk of hypoglycaemic encephalopathy and possibility of developing insulin antibodies on long-term use limit its utility. Oral hypoglycaemic agents also possess some side effects (Chaturvedi **et al.**, 1984).

There is an increasing demand from patients for natural antidiabetic agents. This is more because insulin taken orally has limitations. Oral hypoglycaemic have many side reactions and toxicity besides their ineffectiveness in lowering the blood sugar in chronic stage of diabetes after a certain period (Singh **et al.**, 1985).

The efficacy of leaf powder of **G. sylvestre** in checking glycosuria has already been reported. Experimental studies of this plant also revealed the reduction in blood sugar level in rabbits (Srivastava **et al.**, 1981). Though the plant might be considered to be a boon to patients suffering from diabetes mellitus, it is very unfortunate that it cannot be propagated easily. Also it might be worth noticing that the natural distribution

of the plant is confined to certain places. Considering these problems, attempts were made to produce callus from leaves of *G. sylvestre* under aseptic conditions, so as to isolate the active ingredients from the callus.

MATERIALS AND METHODS

Mature and young leaves of the plant were collected from field – growth 5 year old vines. They were washed first with teepol, then with distilled water and surface sterilized with 0.2% mercuric chloride for 2

minutes and washed with sterile water. The leaf segments were then taken aseptically and inoculated into MS basal medium containing different concentrations of auxin and cytokinin for the initiation of callus. The percentage of callusing, callus growth etc. were recorded after two weeks.

RESULTS AND DISCUSSIONS

Observations on the growth of callus after two weeks are presented in Table 1.

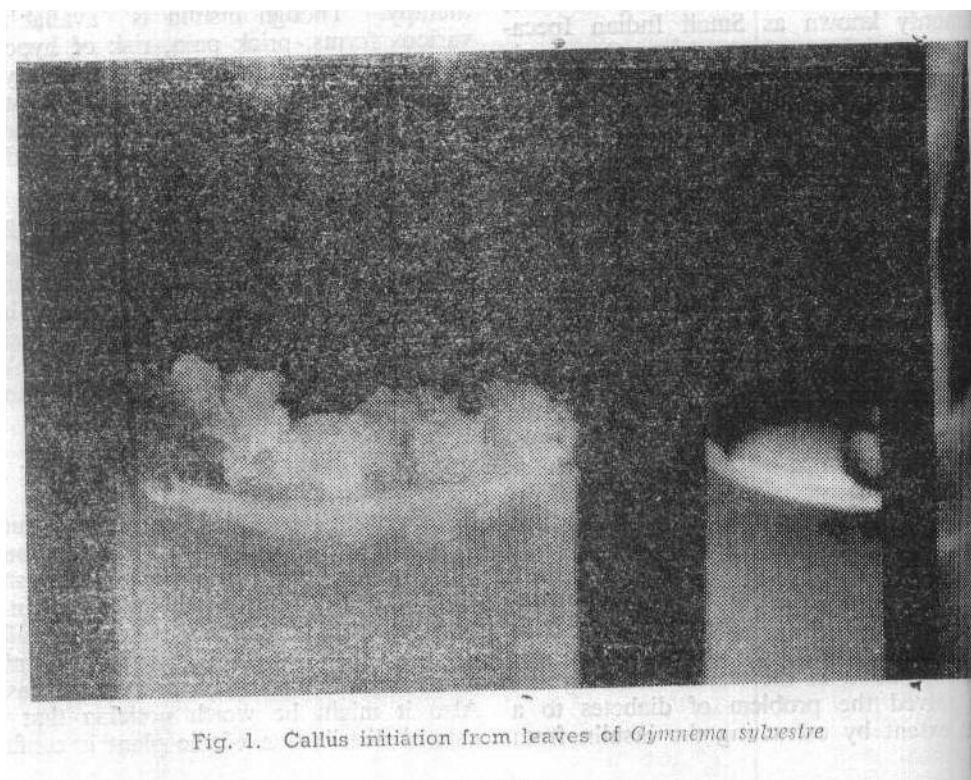
TABLE 1

Callus production from the leaf segments of *Gymnema sylvestre*, two weeks after inoculation

S. No.	Treatments	Young leaf segments			Mature leaf segments		
		Percentage of callusing	Callus growth	Callus index	Percentage of callusing	Callus growth	Callus index
1	MS + 0.5 ppm NAA + 0.5 ppm kinetin	20	1	20	25	1	25
2	MS + 1 ppm NAA + 1 ppm kinetin	100	1	100	100	3	300
3	MS + 2 ppm NAA + 1 ppm kinetin	100	1	100	100	3	300
4	MS alone	-	-	-	-	-	-
5	MS + 2 ppm BA	-	-	-	-	-	-

Amount the various concentrations of auxins and cytokinins tried, MS medium with 1 ppm kinetin, 1 ppm NAA, and with 1 ppm kinetin, 2 ppm NAA were equally effective in producing callus from the leaves (see Fig.1). Response of the older leaves was

found to be more (G = 3, CI = 300) in both the treatments, as compared to the young leaves (G = 1, CI = 100) after two weeks. Yield of callus after frequent sub culturing was also high.



There are few plant tissue and cell cultures which can produce secondary products comparable to that intact plant. Kaul and Staba (1968) have reported the synthesis and isolation of diosgenin from **Dioscorea deltoidea** callus and suspension cultures. Trials on the differentiation of callus and isolation of active ingredients from the callus is in progress.

The prospects of the production of callus are immense. Besides the production of

plantlets, it could be possible to isolate active ingredients from the callus. The useful compound could be produced under controlled environmental conditions, independent of climatic changes or soil conditions. High – yielding strains of cells could be isolated from the callus mass and multiplied further. Automated control of cell growth and rational regulation of metabolic processes would contribute to the reduction of labour cost and improvement of productivity (Tabata, 1977).

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